PATENT

Attorney Docket No. 17761-704

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application	)	Confirmation No. 9677
Inventor: David A. Paslin	)	Art Unit: 1653
Application No: 09/920,897	)	Examiner: ROBINSON, Hope A.
Filed: August 1, 2001	)	
Title: Atopic Dermatitis Treatment Method	)	

DECLARATION UNDER 37 C.F.R. §1.132

I, David A. Paslin, declare as follows:

1. I am the inventor of the patent application identified above and the subject matters described and claimed therein.

2. I am currently a physician, board certified in dermatology (1974) and in dermatopathology (1978), and I am in the active clinical practice of dermatology and dermatopathology at 460 – 34th Street, Oakland, CA, 94609.

3. From 1973 to the current time I have been on the clinical faculty in the Department of Dermatology at the UCSF School of Medicine and currently hold the position of Assistant Clinical Professor. The detail of my education and professional experiences is provided in the attached curriculum vitae of mine.

4. I have reviewed the Examiner's Office Action mailed December 17, 2003. I believe that the disclosure of my patent application as originally filed adequately describes the claimed invention of using MC148P for the treatment of atopic dermatitis (AD), and the claimed invention is not only novel and but also non-obvious over the cited references, Krathwohl et al., Fife et al., Untereker et al., and Fuisz. However, to aid the Examiner's understanding of the claimed invention, I hereby provide supplemental experimental evidence to show that an MC148P can specifically inhibit chemotaxis of AD-relevant cells (4DE4 pre-B lymphoma cells) induced by a specific ligand for CCR8 (I-309). The in vitro experiments further demonstrate that an MC148P can be used for treating AD through suppression of the inflammatory responses of AD that is predominantly mediated via activated Th2 cells expressing CCR8.

5. In vitro Experiments on 4DE4 pre-B lymphoma cells and MC148P: Parental and CCR8-expressing 4DE4 pre-B lymphoma cells were cultured and maintained in selection media (2mg/ml G418). At the time of the experiment, cells were washed in 1X PBS and resuspended in chemotaxis buffer (RPMI medium containing 0.5% BSA) at a final concentration of $5x10^6$ cells/ml. 500,000 cells were placed into the upper wells of a 5 micron disposable transwell chamber (Costar). Five hundred μl of chemotaxis buffer containing various concentrations of recombinant chemokines I-309 (R&D Systems) or SDF-1β (R&D Systems) in the presence or absence of an MC148P were aliquoted into the lower well of the transwell chamber. Cells were incubated at 37°C and allowed to chemotax into the lower chamber for approximately 3 hours. Chemotaxed cells reaching the lower chamber were collected and counted via flow cytometry at a set time of 1 minute. Parental and CCR8-expressing 4DE4 cells were tested side-by-side for dose responsiveness to I-309.

6. Results from Applicant's Experiments on 4DE4 pre-B lymphoma cells: Tables I-III summarize results obtained in experiments on chemokine-induced chemotaxis of parental and CCR8-expressing 4DE4 pre-B lymphoma cells in the presence and absence of MC148P.

Table I. Effects of CCR8-Specific Ligand I-309 on Chemotaxis of Parental and CCR8-Expressing 4DE4 cells

	Chemotaxis of Parental 4DE4 Cells (cells/min required)	Chemotaxis of CCR8-expressing 4DE4 cells (cells/min required)
0 nM of I-309	113	113
0.1 nM of I-309	158	232
1 nM of I-309	138	505
10 nM of I-309	143	2256
100 nM of I-309	145	5185

Table II. Effects of MC 148P on Chemotaxis of CCR8-Expressing 4DE4 cells in the Presence of a CCR8 Specific Ligand, I-309

	Chemotaxis of CCR8-Expressing 4DE4 cells (+ 10 nM I-309) (cells/min required)
0 nM MC 148 protein	1861
2 nM MC 148 protein	661
10 nM MC 148 protein	611
50 nM MC 148 protein	219
150nM MC 148 protein	428

Table III. Effects of MC 148P on Chemotaxis of CCR8-Expressing 4DE4 cells in the Presence of a CCR8 Non-Specific Ligand, SDF-1β

	Chemotaxis of CCR8-Expressing 4DE4 cells (+ 50 nM SDF-1β) (cells/min required)
0 nM of MC148P	4857
2 nM of MC148P	5082
10 nM of MC148P	4863
50 nM of MC148P	3594
150 nM of MC148P	3456

As shown in Table I, I-309, a specific ligand for CCR8, had no detectable effect on chemotaxis of **parental** 4DE4 pre-B lymphoma cells, while it induced measurable and dose-

dependent chemotaxis of **CCR8-expressing** 4DE4 pre-B lymphoma cells in the range of 1-100 nM with an estimated EC_{50} of approximately 10 nM. As shown in Table II, CCR8-expressing 4DE4 cells stimulated with I-309 (10 nM) exhibited dose-dependent inhibition by MC 148P with an estimated IC_{50} of approximately 2 nM. In contrast, CCR8-expressing 4DE4 cells stimulated with a non-specific ligand for CCR8, SDF-1β (50 nM), exhibited only partial dose-dependent inhibition by MC 148P with an estimated IC_{50} of >150 nM (Table III).

6. Comparison of Applicant's Experiments with the Experiments described in Krathwohl et al: There are fundamental differences between the chemotactic experiments of Krathwohl et al (Proc Natl Acad Sci 94:9875-80, 1997) and Applicant's chemotactic experiments. In brief, Krathwohl et al. used mononuclear cells obtained from venous blood of healthy human volunteers and stimulated chemotaxis of these diverse cells with MIP-1α, a CC (β) chemokine. Although inhibition of MIP-1α stimulated chemotaxis was obtained with MC 148p, there was no attempt by Krathwohl et al. to identify either the subtypes of cells subject to MC 148p mediated inhibition of MIP-1α stimulated chemotaxis or to identify the specific receptors on the cells mediating these effects.

MIP-1α is a ligand for CCR1, CCR4, CCR5 and the CMV receptor US28 (Proudfoot AEI. Immunol Rev 177:246-256, 2000). CCR1 is found on T cells (types not specified) and eosinophils whereas CCR5 is found on both Th1 and Th2 cells in some experiments, but at higher levels on Th1 than on Th2 cells in other experiments as well as on monocytes (O'Garra A. Current Biology 8:R646-649, 1998), but a review of deletion experiments suggests primarily Th1 effects for both CCR1 and CCR5 (Proudfoot AEI. Immunol Rev 177:246-256, 2000). These data suggest that it was mostly Th1 cells and monocytes that were attracted by MIP-1α and inhibited by MC 148p in the Krathwohl experiments, which has little to no relevance to Atopic Dermatitis (AD) which is predominantly mediated via Th2 cells. By contrast, Applicant's experiments used cells expressing CCR8 similar to cells found in the lesional skin of patients with AD (Biedermann T. Eur J Immunol 32: 3171-80, 2002). In this manner, Applicant's experiments have direct relevance to the in vivo pathogenesis and treatment of patients with AD; the Krathwohl experiments do not.

Published literature further supports Applicant's view that the Krathwohl experiments that used peripheral blood of healthy volunteers fails to teach or suggest using MC148P for treating AD. Of the 4 receptors for which MIP-1α is a ligand, only CCR4 is characteristically expressed on Th2

cells. Yet of the CCR4+ cells in normal human peripheral blood, approximately 80% make neither Th1 nor Th2 cytokines, 11% make Th1 cytokines and 9% make Th2 cytokines (Kim CH. J Clin Invest 108: 1331-39, 2001). The cytokine profiles of the cells used in the Krathwohl experiments reflect the percentage profiles for peripheral blood of healthy volunteers, but do not represent the Th2 mediated inflammatory infiltrate of patients with AD in whom activated Th2 cells are predominant. Given that only about 9% of CCR4+ cells in normal human venous blood make Th2 cytokines, inhibition of chemotaxis of CCR4 expressing cells has little bearing on the MC 148p mediated inhibition of chemotaxis shown in the Krathwohl experiments. It is highly unlikely that the peripheral venous blood mononuclear cells from normal volunteers, as used in the Krathwohl experiments, contained any activated Th2 cells expressing CCR8 (Colantonio L. Eur J Immunol 32: 1264-1273, 2002).

It is Applicant who realized that MC148P inhibits inflammation of AD patients mediated by activated Th2 cells and designed in vitro experiments to further confirm the clinical data. In the involved skin of AD, the perivascular infiltrate of T cells are activated (Cooper KD. JAAD 45:S10-S12, 1999) and activated Th2 cells are present and predominant. It is these activated Th2 cells, known to express CCR8, that fail to appear in the presence of MCV papules as documented in the clinical and microscopic photos of AD as provided and described in this patent application. Applicant's in vitro experiments demonstrate that the parental line of 4DE4 pre-B lymphoma cells, lacking expression of CCR8, are not stimulated by I-309, a specific ligand for CCR8. The experiments also show that the 4DE4 cells expressing CCR8 are stimulated by I-309 and that the chemotaxis so induced is inhibited by MC148P in a dose-dependent manner, indicating that MC148P binds to CCR8 and blocks activation of CCR8 thereby preventing I-309 stimulated chemotaxis of these cells. Since I-309 is produced by IL-1 stimulated monocytes, activated T cells and activated mast cells (Luttichau HR. J Exp Med 191: 171-179, 2000), I-309 induced chemotaxis is directly relevant to the inflammatory state of patients with AD. Thus, Applicant's experiment on specific inhibition of I-309 induced chemotaxis by MC148P is directly relevant to suppression of inflammation in patients with AD.

7. Conclusion: In summary, Applicant's experiments demonstrate that inhibition of chemotaxis of CCR8-expressing cells in vitro directly corresponds to the inhibition of chemotaxis seen in AD in vivo as shown in the clinical and microscopic photos provided and described in the

instant patent application. These lines of evidence further support the claimed invention of using MC148P for treating AD patients. None of the cited reference teaches or suggests targeting CCR8-expressing Th2 cells using MC148P, let alone teaching or suggesting treating AD or other Th2-mediated diseases using MC148P. Thus, Applicant believes that the claimed invention is adequately described in the application as originally filed, and is not only novel but also non-obvious over the cited references.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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CURRICULUM VITAE OF DAVID A. PASLIN, M.D.

Academic Training

Stanford University	A.B. degree	1958-62
UCLA School of Medicine	M.D. degree	1962-63 1964-67
Harvard Law School		1963-64
USPHS Hospital, Staten Island	Straight Medical Internship	1967-68
USPHS Hospital, Staten Island	General Medical Officer	1968-70
University of Pennsylvania	Dermatology Residency	1970-73

Academic Appointments

Clinical Instructor of Dermatol	UCSF School of Medicine	1973-80
Ass't Clinical Prof of Dermatol	UCSF School of Medicine	1980-Present

Honors

Honors at entrance to Stanford University	1958
Graduation with distinction from Stanford University	1962
Nelson Paul Anderson Memorial Essay Award of the Pacific Dermatologic Association	1973

Board Certification

Dermatology	1974
Dermatopathology	1978

Scientific Publications

Paslin DA & Heaton CL. Acral arteriolar ectasia. Arch Derm 106: 906-908, 1972.

Paslin DA. The effects of depigmenting agents on the growth of a transplantable hamster melanoma. Acta Dermatovener 53: 119-122, 1973.

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Paslin DA, Heaton CL, Gray Wood M. Scar biopsy in sarcoidosis. Dermatologica 146: 315-319, 1973.

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Paslin D, Dimitrov NV, Heaton C. Regression of a transplantable hamster melanoma by intralesional injections of corynebacterium granulosum. J Natl Can Inst 52: 571-573, 1974.

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Paslin D. Cartilaginous papule of the ear. J Cutan Pathol 18: 60-63, 1991.

Paslin D. Treatment of lichen sclerosus with topical dihydrotestos-terone. Obst & Gyn 78: 1046-1049, 1991.

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Manuscripts Submitted for Publication

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Paslin D & Wertz P. A study to determine the effect of tacrolimus on ceramide levels in the stratum corneum of patients with atopic dermatitis, 2003.

Hill J, Paslin D, Wertz PW. A new covalently bound ceramide from human stratum corneum--ω-Hydroxyacylphytosphingosine. 2004.

Books Published

Paslin DA. The Hide Guide. Celestial Arts (Ten Speed Press) pages 1-285, 1981.

Chapters Published

Paslin DA. The porphyrias. Pages 717-727. In Current Diagnosis 9. Edited by Conn RB, Borer WZ, Snyder JW. Saunders Company. Philadelphia 1997.

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Comments on dermatology at the University of Pennsylvania. Pages 171-172. In Beerman H & Lazarus GS: The Tradition of Excellence. 1986.

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Patents

Method and system for growing molluscum contagiosum virus in xenografts to immunocompromised hosts. Filed August 20, 1996. Issued as U.S. Letters Patent No. 5,885,822 on March 23, 1999.

Atopic dermatitis treatment method. U.S. Patent Application Serial No; 09/920,897, Filed August 1, 2001.

Letters Published

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Paslin DA. Keratinocytic vs. neutrophilic causation in psoriasis. Int J Dermatol 30: 601, 1991.

Occupation:

Private practice in dermatology, Oakland, California.